



PATENT  
ATTORNEY DOCKET NO. 051501-0305443

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant : Michael Croft, et al.                      Art Unit : 1644  
Serial No. : 10/661,358                                  Examiner : Ouspenski, Ilia  
Filed : 09/11/2003  
Title : METHODS OF TREATING OX40 MEDIATED RECALL IMMUNE  
RESPONSES

Assistant Commissioner for Patents  
Washington, DC 20231

**DECLARATION UNDER 37 C.F.R. §1.131**

Dear Sirs:

We, Dr. Michael Croft, and Dr. Shahram Salek-Ardakani, do hereby declare and state that:


1. We are the inventors of the subject matter described and claimed in United States Patent Application Serial No. 10/661,358, filed September 11, 2003, entitled: "METHODS OF TREATING OX40 MEDIATED RECALL IMMUNE RESPONSES."
2. We are familiar with the prosecution history of Application Serial No. 10/661,358.
3. We understand that the Examiner has cited Arndt *et al.* (U.S. Patent Application Publication No. 2004/0009174 A1) under 35 U.S.C. §102(e) and §103(a) against claims 1 to 9, 11, 15 to 20, 23 to 39, and 69 to 76 of Application Serial No. 10/661,358.
4. We submit that Arndt *et al.* (U.S. Patent Application Publication No. 2004/0009174 A1) is not available as prior art under either of 35 U.S.C. §§102 and 103.
5. We were diligent from the time that claims 1 to 9, 11, 15 to 20, 23 to 39, and 69 to 76, as presented in the accompanying Response were conceived prior to the December 18, 2001, filing date of the priority application of Arndt *et al.* (Application Serial

No. 60/341,453), and up until the September 11, 2002, filing of provisional patent application serial no. 60/410,534, to which this application claims priority.

6. Evidence of conception is supplied in the form of copies of four pages from our laboratory notebooks (Exhibit A, dates redacted), each of which is labeled 1 to 4.
7. In particular, studies were of mice (BL/6) sensitized with 20 ug OVA/Alum to induce asthmatic lung inflammation, and subsequently treated or untreated with anti-OX40L antibody. In brief, three groups of four mice, Groups A, B and C, (page 1) were immunized with OVA (day 0). Following immunization, Group A control mice were administered 150 ug rat IgG for 3 days (days 24-27), Group B mice were administered 150 ug anti-OX40L antibody for 3 days (days 24-27), and Group C mice were administered 150 ug anti-OX40L antibody for 4 days (days 27-31) (page 1). Mice were challenged via the airways in a recall immune response with OVA (5 mg/ml) delivered in aerosol for 30 minutes each day during days 28-31. Mice were sacrificed after the last challenge and airway measurements performed to ascertain lung inflammation.
8. Cytokines, namely IL-4, IFN, IL-5 and IgE, were measured in lung lavages (see, "OX40L blocking Asthma," pages 2 and 4). IL-4, which is typically increased in asthma, was reduced by an average of approximately 4-fold in the anti-OX40L antibody treated Groups B and C mice (Avg 0.1965 and 0.228), compared to control Group A mice (Avg 0.884). IgE levels, which also typically increase in asthma (pages 2 and 4) was also reduced in anti-OX40L antibody treated Groups B and C mice (Avg 942), compared to control Group A mice (Avg 1229). These studies therefore indicate that anti-OX40L antibody reduces clinical indicia of asthma.

9. Neutrophil, eosinophil, monocyte and lymphocyte numbers were also measured in lung lavages (pages 3 and 4). Eosinophil and Lymphocyte numbers and percentages are typically increased in the asthmatic lung (page 13, lines 22-29 of the specification). Eosinophils were reduced in the lung of anti-OX40L antibody treated Groups B and C mice (Avg 14% and 20%;  $1.11 \times 10^4$  and  $1.66 \times 10^4$  cells) compared to control Group A mice (Avg 37% total;  $5.16 \times 10^4$  cells). Lymphocytes were also reduced by an average of approximately 6-fold in the lung of anti-OX40L antibody treated Groups B and C mice (Avg 1.3% and 1.1% total cells;  $0.11 \times 10^4$  and  $0.09 \times 10^4$  cells) compared to control Group A mice (Avg 7.8% total cells;  $0.64 \times 10^4$  cells). These studies therefore indicate that anti-OX40L antibody reduces eosinophil and lymphocyte infiltration of lung associated with asthma.
10. We declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 2.28.06

  
Michael Croft, Ph.D.

Date: 2.28.06

  
Shahram Salek-Ardakani, Ph.D.

12 B6/6 mice immunized with 90  $\mu$ g  
OVA / Alum.

Group A: injected with 150  $\mu$ g  $\alpha$ 0X40L 156 day 21-2  
3 days.

Cage # AE 6513

Group B: injected with 150  $\mu$ g  $\alpha$ 0X40L 3.  
day 24-27, Cage # AE 6512

Group C: injected with 150  $\mu$ g  $\alpha$ 0X40L, 4.  
day 27-31, Cage # AE 6511

Aerosol (5 mg/ml OVA) 10 ml ~ 30 min  
(4 days).

	24	25	26	27	28	29	30	31
A I. 150 $\mu$ g IgG								
B II. 150 $\mu$ g $\alpha$ 0X40L								
C III. 150 $\mu$ g $\alpha$ 0X40L								
Aerosol (5 mg/ml OVA)								
Airway								

OX40L blocking Asthma

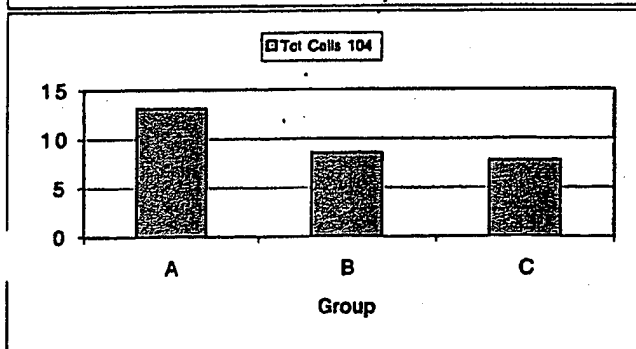
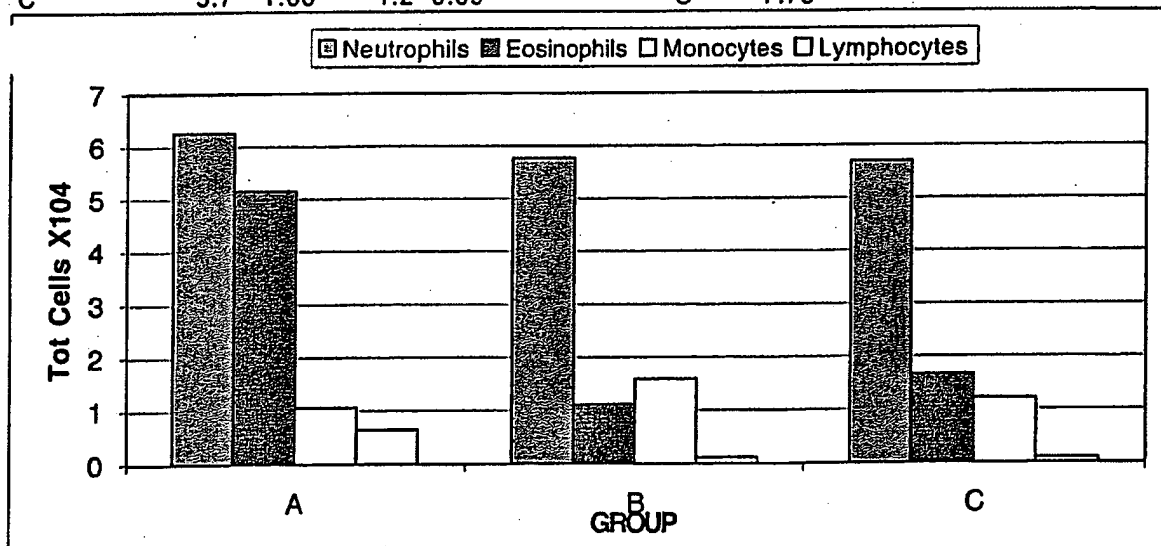
Group A	IL-4(ng/ml)	IFN	IL5	IL13	IgE (ng/L)	
1	1.52 <1.0		<0.3		1147.5	
2	0.248 <1.0		<0.3		997.2	
3	<0.1 <1.0		<0.3		1377.8	Control
4	<0.1 <1.0		<0.3		1395.8	
Avg	0.884				1229.575	
Group B						
1	0.35 <1.0		<0.3		693.7	
2	0.046 <1.0		<0.3		734.4	2.07402
3	0.182 <1.0		<0.3		1537.2	2.1437
4	0.208 <1.0		<0.3		803.2	
Avg	0.1965				942.125	
Group C						
1	0.22 <1.0		<0.3		305.7	
2	0.304 <1.0		<0.3		867.7	
3	0.16 <1.0		<0.3		1240.12	2.07402
4	<0.1 <1.0		<0.3		1356.4	2.0730
Avg	0.228				942.48	

# Differential cell counts

Differential cell counts														
Group A		Neutrophils			Eosinophils			Monocytes			Lymphocytes			Tot cells10 <sup>4</sup>
		#	%	Tot # of	#	%	Tot # of	#	%	Tot # of	#	%	Tot # of	
		cells X104			cells X104			cells X104			cells X104			
1	83	62.4	9.36	44	33.1	5	6	4.5	0.7	0	0	0	15	
2	57	43.2	9.07	62	47	9.87	10	7.6	1.6	3	2.2	0.46	21	
3	53	44.9	4.27	51	43.2	4.1	11	9.3	0.88	3	2.6	0.25	9.5	
4	14	33.3	2.33	11	26.2	1.8	6	14.3	1	11	26.2	1.83	7	
Average		46	6.26		37	5.19		8.9	1.05		7.8	0.64	13.125	
Group B														
1	76	72.4	5.07	19	18.1	1.27	10	9.5	0.67	0	0	0	7	
2	58	54	3.83	25	23.6	1.65	2	18.9	1.4	3	2.8	0.2	7	
3	73	67	7.5	12	11	1.23	24	22	2.46	0	0	0	11.2	
4	89	74.2	6.68	4	3.3	0.3	24	20	1.8	3	2.5	0.23	9	
Average		67	5.77		14	1.11		18	1.58		1.3	0.11	8.55	
Group C														
1	33	55	5.5	7	11.7	1.17	20	33.3	3.3	0	0	0	10	
2	5			5			3			6			5	
3	78	66.1	4.76	37	31.4	2.26	3	2.5	0.18	0	0	0	7.2	
4	98	77.8	6.85	22	17.5	1.54	2	1.6	0.14	4	3.2	0.28	8.8	
Average		66	5.7		20	1.66		13	1.2		1.1	0.09	7.75	

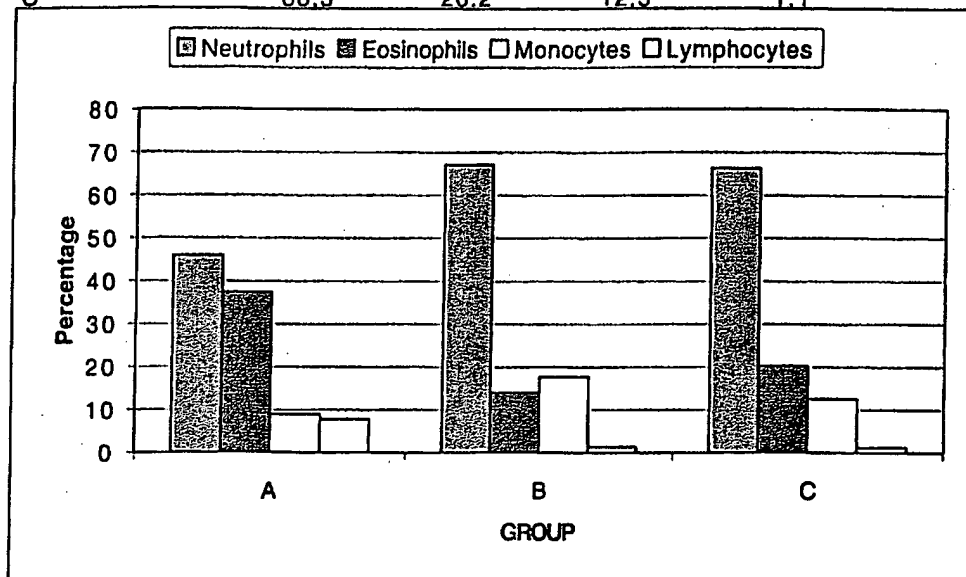
Average cell #

GP	Neutr	Eosino	Monocyt	Lymphocytes	Tot Cells 104
A	6.3	5.16	1.05	0.64	13.1
B	5.8	1.11	1.58	0.11	8.55
C	5.7	1.66	1.2	0.09	7.75



# Average Differential cell counts

	Neutrophils	Eosinophils	Monocytes	Lymphocytes
A	45.95	37.4	8.9	7.8
B	67.1	14	17.6	1.3
C	66.3	20.2	12.5	1.1



## Total IgE

	A	B	C
1	1147.5	693.7	305.7
2	997.2	734.4	867.7
3	1377.8	1537.2	1240.1
4	1395.8	803.2	1356.4
Mean	1229.575	942.125	942.475

